POLYBUFFER SEPARATION OF THE ALKALOIDS OF *Nitraria schoberi*

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By extracting the epigeal part of *Nitraria schoberi* L., collected in the Kyzylkum, Bukhara oblast in the budding period we have obtained 0.45% of ether-soluble and 0.18% of chloroform-soluble alkaloids.

The bulk of the main alkaloid of the ether-soluble fraction — nitrarine [1] — was isolated by its direct treatment with acetone. The acetone mother liquor and the chloroform-soluble fraction of the alkaloids consisted of a complex mixture of bases which was impossible to separate by chromatography on columns containing absorbents, by the preparation of salts, by solubilities, and by the other usual methods. Consequently, we made an attempt to fractionate the mixture of alkaloids by the polybuffer separation method [2] in an apparatus for automatic liquid extraction [3].

The material from the acetone mother liquor after the separation of the nitrarine and evaporation to dryness was dissolved in chloroform to form a 5% solution, and this was passed through a series of phosphate buffer solutions with different pH values arranged in order of decreasing pH value.

TABLE 1. Results of the Separation of the Ether-Soluble and Chloroform-Soluble Bases

Fraction	pH of the buffer	Weight of the fraction, g		
		ether extract	chloroform extract	chloroform phase
Ether-soluble fraction				
1	Distilled water	45,8		71,7
2	9,0	18,8	4,0	27,9
3	8,0	50,8	12,4	47,4
4	7,5	48,6	15,4	42,2
5	7,0	108,2	33,0	
6	6,5	33,7	9,6	
7	6,0	47,7	9,3	38,1
8	5,5	28,2	6,1	
9	5,0	21,9	8,0	
10	4,0	16,5	3,8	
11	3,0	27,0	6,3	16,9
12	2,0	39,4	18,3	
Total		486,6	126,2	244,2
Chloroform-soluble fraction				
13	Distilled water	66,4		
14	9,0	0,6	2,2	
15	8,0	3,4	10,2	
16	7,0	15,1	23,4	
17	6,0	14,5	13,7	24,1
18	5,0	11,5	6,4	
19	4,0	4,7	5,2	
Total		116,2	61,1	24,1

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Each column was previously charged with 700 ml of chloroform to fill the side tubes, and then the first five columns were filled with distilled water and each of the subsequent columns was filled with 1.5 liters of buffer solution with a definite pH value (from 9 to 2).

The residue (957 g) of the ether-soluble alkaloids after the separation of the nitrarine was divided into three parts (307, 300, and 350 g), each of which separately was passed in the form of a 5% solution in chloroform as described above through five columns containing distilled water and through buffer solutions with pH values of 9 to 7 (four columns each). The residues after the column with pH 7 from all three parts (35% of the total amount) of the ether-soluble fraction were combined and subjected to further separation in an apparatus into fractions with pH values from 6.5 to 2 (3 columns each).

After the solution had been passed through all the columns, washing with chloroform was continued. The solution of the mixture of alkaloids and the chloroform were passed at the rate of one liter/h. After the end of separation, the buffer solutions and the chloroform phases were discharged from the column separately, and then the fractions with the same pH values were combined. The buffer solutions were made alkaline with 25% caustic soda solution and extracted exhaustively first with ether and then with chloroform. In this way we obtained 12 ether-soluble and 12 chloroform-soluble fractions. The extracts were evaporated to dryness. Table 1 gives quantitative information on the separation of the original ether-soluble alkaloids.

The largest amount of alkaloids was present in fractions 1-5 (65%), which shows the predominance of alkaloids of medium and high basicity in the mixture. After passage through the apparatus, a total of 857 g of the total alkaloids was recovered, i.e., out of the 957 g of the original ether-soluble fraction 100 g consisted of ballast substances and the true yield of the original ether-soluble fraction was 0.4% of the weight of the dry raw material.

The original chloroform-soluble fraction of the alkaloids of *Nitraria schoberi* was separated similarly. From 411 g of the combined alkaloids a 5% solution in chloroform was prepared and it was passed through the apparatus (pH 9-4) in two portions. The first four columns were filled with distilled water, the next two with a buffer solution have pH 9, and all the subsequent buffer solutions with pH values from 8 to 4 were contained in three columns each.

Only half the initial chloroform fraction was recovered after passage through the apparatus, and the remainder of it apparently consisted of ballast substances of nonalkaloid nature (see Table 1).

Thus, the epigeal part of *Nitraria schoberi* contained 0.5% of total alkaloids on the weight of the dry raw material. Below we give figures on the percentage distribution of the mixture of alkaloids relative to the total amount of bases and to the weight of the dry plant (the chloroform phases from the columns are not given; they were originally collected separately and subsequently part of them was combined):

Fraction*	% of Total	% on the Plant	Fraction	% of Total	% on the Plant
1	4.3	0.0200	10c	0.4	0.0018
2e	1.8	0.0085	11e	2.5	0.0123
2c	0.4	0.0018	11c	0.6	0.0027
3e	4.8	0.0230	12e	3.7	0.0170
3c	1.1	0.0054	12c	1.7	0.0083
4e	4.6	0.0220	13	6.2	0.0297
4c	1.4	0.0067	14e	0.1	0.0004
5e	10.2	0.0480	14c	0.2	0.0008
5c	3.1	0.0150	15e	0.3	0.0012
6e	3.1	0.0150	15c	1.0	0.0045
6c	1.0	0.0045	16e	1.4	0.0067
7e	4.5	0.0210	16c	2.3	0.0100
7c	0.9	0.0043	17e	1.4	0.0065
8e	2.6	0.0126	17c	1.3	0.0063
8c	0.6	0.0027	18e	1.1	0.0054
9e	2.1	0.0100	18c	0.6	0.0027
9c	0.8	0.0036	19e	0.5	0.0022
10e	1.5	0.0072	19c	0.5	0.0022

*e) Ethereal extract; c) chloroform extract.

From the various fractions obtained on the apparatus we isolated and studied nine alkaloids (Table 2).

TABLE 2. Alkaloids of *Nitraria schoberi* L.

Alkaloid	mp, °C	Comp.	Mol. wt.	Derivatives, mp, °C	Fraction*	Literature
Nitramine	Oil	$C_{10}H_{19}NO$	169	Hydrochloride (HC) 205–207 Nitrate, 204-206	3 e 2 e	[4]
Nitrarine	256–257	$C_{20}H_{25}N_3$	307	HC, 265–267	5 e 16e	[1]
Isonitrarine	208–209			HC, 238–239	5 e	[5]
Nitramidine	Amorph.	$C_{20}H_{23}N_3$	305	HC, 251–253	5 e	[6]
Schoberidine	204–205	$C_{20}H_{21}N_3$	303	HC, 266–267	3 c	[7]
Base A	—	—	232	—	3 e	[8]
Nitraramine	85–86	$C_{15}H_{24}N_2O$	248	HC, 219–220	6 e 7 e	[9]
Schoberine	60–61	$C_{15}H_{26}N_2$	234	Picrate, 182–184	10 e chloroform phase of fractions 5-12)	[10]
Base B	264–265	$C_{20}H_{24}N_2O$	308	Acetate, dihydro		[8]

*e) Ether extract; c) chloroform extract.

Nitraramine in the form of the nitrate was isolated from the ethereal extracts of fraction 2 (2e) and 3 (3e). Fraction 3c yielded schoberidine in the form of the perchlorate.

The largest amount of alkaloids was isolated from fraction 5e — isonitrarine, nitrarine, and nitramidine. They were separated according to the solubilities of their hydrochlorides in ethanol. The main alkaloid of this fraction was isonitrarine. The ethereal extract of the pH 8 fraction (3e) yielded a small amount of a base A, a larger amount of which will be necessary for its complete characterization.

Fractions 6e and 7e yielded the alkaloid nitraramine [9] in the form of the nitrate. Schoberine [10] and base B [8] were isolated from fraction 10e and from the combined chloroform phases 5-12.

EXPERIMENTAL

The KSK silica gel taken for thin-layer chromatography was ground in a ball mill and sieved, the fraction with a grain size of 90-71 nm being taken. The sorption mass was prepared by mixing the silica gel with a saturated solution of calcium sulfate. In addition, alumina was used for TLC.

The spots were revealed with iodine vapor and with Dragendorff's solution.

For TLC we used the following main solvent systems: chloroform–methanol (9:1 and 19:1); chloroform–acetone–diethylamine (5:4:1); chloroform–diethylamine (9:1); chloroform–benzene–methanol (5:4:1); and chloroform–ethanol (2:1).

The alkaloids were extracted from the plants with a 2% solution of acetic acid in chloroform. The buffer solutions were prepared by mixing a 2% solution of KOH and orthophosphoric acid.

SUMMARY

It has been established that the epigeal part of the plant *Nitraria schoberi* L. in the budding stage contains 0.5% of alkaloids.

By a combination of the fractionation of the mixture of alkaloids by polybuffer separation with other methods of separation, nine alkaloids have been isolated and studied.

Fractions with a high pH value yielded the following strong bases: nitramine (pH 9 and 8), schoberidine and base A (pH 8), and nitrarine, isonitrarine, and nitramidine (pH 7). The following alkaloids were also isolated: nitraramine (pH 6.5 and 6) and schoberine and base B (pH 4 and the combined chloroform phases of the fractions with pH 7-2).

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SYNTHESIS OF PROTECTED OLIGOPEPTIDES REPRESENTING FRAGEMENTS OF HISTONE FRACTION HI

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The functional role of the primary structure of histones may be shown both in interaction with such specific enzymes as, for example, phosphokinase [1, 2] and also in the process of forming complexes with DNA [3]. Interesting information can be obtained in the investigation of individual fragments of histones. We have previously reported the isolation of compounds of this type [4]. The present paper describes the synthesis of a number of analogous oligopeptides forming segments of the histone fraction HI of calf and rabbit thymus. It must be noted that there are some differences between these histone fractions. Thus, in the HI of rabbit thymus the Ala-38 residue is replaced by Ser-38. This determined the type of compounds obtained: the fragment of the HI of rabbit thymus (31-40) contained Ala-38 and the fragment of the calf thymus HI (31-41) contained Ser-38. In addition, we synthesized two analogs in which L-Ala-37 was replaced by D-Ala-37, and the Pro-Pro fragment by Ala-Ala. In contrast to the natural sequence, in a number of oligopeptides a Lys residue was present in place of Arg-35.

The synthesis of the oligopeptides was effected in stages from the C-end by blocks consisting of 2-3 amino-acid residues using the method of mixed anhydrides and the carbodiimide method according to Schemes I and II. It must be observed that during the synthesis of the peptides the possibility of partial racemization is not excluded. In view of this, we performed the synthesis in such a way that, as far as possible, the C-terminal residue was a glycine residue. In this way, we obtained the peptide with sequence (31-40) by a (2 + 9) scheme. If the C-terminal residue was an optically active amino acid, we used the carbodimide method with tetrahydrofuran as solvent. The reaction mixture was maintained at 0 to −6°C for 1 h and at 22°C for 20 h. Under these conditions, according to the literature [5], racemization does not exceed 0.1%.

The N^{ε}-amino group of glycine was protected by a benzyloxycarbonyl grouping (Z), and the N-α-amino groups of the acids by a tert-butoxycarbonyl (Boc) grouping. The guanidine groups of the arginine residues were protected by nitro groups (NO_2).

The compounds obtained are stable and are being used as the starting materials for the preparation of the methyl esters of these peptides.

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